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# COMPARISON OF HbA1c LEVEL BETWEEN IRON DEFICIENCY ANEMIC NON-DIABETIC & NON ANEMIC NON-DIABETIC SUBJECTS

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## ABSTRACT

**Objective:** This study was conducted to determine the mean HbA1c levels in non-diabetic iron deficiency anaemic patients & non anaemic non diabetic subjects & to compare the HbA1c in both the groups.

**Material & methods:** It was a hospital based case control analytical type observational study. The case group includes 100 non diabetic iron deficient anemic patients & the control group includes 100 non diabetic non anemic subjects admitted in hospital .The mean HbA1c level was calculated in both the groups & compared & its correlation with various variable like serum ferritin level, serum iron level serum transferrin saturation, was calculated. The data were analysed by chi-square test & T-test & Carl-Pearson correlation coefficient with SPSS version 16 for application.

**Results:** The mean HbA1c level in iron deficient patients (5.97±0.69%) was significantly higher than in healthy persons (5.24±0.49%). Patients having iron deficiency also had a significantly higher frequency (76/100 cases) of higher HbA1c (>5.5%) compared to healthy persons (32/100 persons). In control group mean HbA1c level in patients having HbA1c level > 5.5 was 5.82 + 0.22 while that in case group was 6.27+ 0.46 with p-value < 0.001 which is statistically highly significant. There was no statistically significant difference in mean fasting plasma sugar & post prandial plasma sugar in case & control group.

The serum iron, serum ferritin & serum transferrin saturation were negatively correlated with HbA1C level in both the groups but only in case group the negative correlation between serum iron & HbA1c level ,& negative correlation between serum ferritin & HbA1c level were more strong & statistically significant .(p value<0.05).

**Conclusion:** The mean HbA1c level is shifted to higher range in iron deficient anaemic non diabetic patients in comparison to non-anaemic non diabetic patients independent of blood sugar level. So HbA1c level should be carefully interpreted in presence iron deficiency anaemia. HbA1c may increase independent of plasma sugar level. Further study is required to explore cause-effect relationship between iron deficiency anaemia & HbA1c.

**KEY WORDS:** Iron deficiency anaemia, HbA1c level, non-diabetes status

## INTRODUCTION:

Iron deficiency anemia (IDA), a major public health problem in developing countries, affects 50 % of world population. IDA is diagnosed by simple RBC indices like MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin) PBF (peripheral blood film) examination showing microcytic hypochromic morphology, serum iron level, serum ferritin level , serum transferrin saturation.

HbA1c is commonly used diagnostic & monitoring tool in diabetes mellitus, reflects average blood sugar level within last 8-12 weeks[3] & its importance came was highlighted in the diabetes control & complication trial (DCCT)[1] & the United kingdom prospective diabetes study (UKPDS)[2]. ADA criteria of HbA1c level for diagnosing diabetes mallitus is $\geq$ 6.5 %. HbA1c level between 5.7 – 6.4 is termed as prediabetes, increased risk for diabetes (ADA), intermediate hyperglycemia (WHO). HbA1c level $\leq$ 5.6 is considered normal. Formed by nonenzymatic irreversible glycation of valine amino acid at N- terminal of beta chain of hemoglobin[4] & is not affected by transient alterations in blood sugar level and is measured by three methodologies:- 1) chromatography; 2) boronate affinity assay; 3) immunoassay

Factors which affect HbA1c level independent of blood sugar level may be due to alteration in glycation i.e. increased or decreased (alcohol, intra-erythrocyte pH, chronic renal failure vit. C & E, hemoglobinopathies etc), alteration in lifespan of RBC i.e. increased or decreased (after splenectomy, splenomegaly antiretrovirals, ribavarin, dapsone hemoglobinopathies etc), factors which affect erythropoisis i.e. increased or decreased erythropoisis (vit $B_{12}$ deficiency, iron deficiency, administration of erythropoietin, chronic liver disease )& finally factors which depends upon measuring methods of HbA1c[5-10]. The techniques using immunoassay methodology is not affected by HbD or HbE, carbamylated hemoglobin & little affected by HbF & relatively easy to perform.[5]

In many studies relationship between IDA & HbA1c level was found both in diabetic & non diabetic patients independent of hyperglycemia & iron deficiency anemia was associated with increased HbA1c level.[11,12,13,14]. Consistent with these observations, iron replacement lowers HbA1c level in diabetic as well as non diabetic patients[11,12,13]. HbA1c level is increased in late pregnancy due to iron deficiency.[14] This may lead to misdiagnosis of diabetes mallitus in non diabetic patients & misinterpretation of HbA1c level in monitoring of sugar control in diabetic patients.

Insight into the mechanism was recently obtained by the observation that malondialdehyde, which is increased in IDA patients[13], enhances the glycation of Hemoglobin[15]. Another proposed mechanism is decreased erythropoisis.

## MATERIAL & METHODS:

### Study Design:

This was hospital based case- control analytical type observational study after approval from Institutional Ethical Committee. This study was conducted on 100 non diabetic non anemic patients (as control group) & 100 non diabetic iron deficiency anemic patients (as case group) admitted in general medicine wards & attending the OPD of general medicine in SMS hospital, Jaipur (Rajasthan).

After taking detailed history , through clinical examination was done. After overnight fasting blood samples of the patient were withdrawn & sent in laboratory for FBS (fasting blood sugar ), complete blood count, PBF( peripheral blood film), MCV, reticulocyte count, serum iron, serum TIBC (total iron binding capacity ), serum ferritin, transferrin saturation, serum creatinine, serum urea. Reticulocyte production index was calculated. PPBS ( post prandial blood sugar) was measured after 2 hour of oral intake of 75 gm anhydrous glucose. Plasma sugar was measured by glucose oxidase method on an automated analyzer. HbA1c was measured by using Quantia HbA1c method which is turbimetric immunoassay for direct determination of HbA1c & is based on the principle of agglutination reaction. This is comparable (correlation coefficient (r) was 0.991) to standardized method according to approved IFCC reference method.

### Inclusion Criteria for Case Group:

Iron deficiency anemia & Non-diabetic patients.

### Criteria for Iron Deficiency Anaemia:

Haemoglobin < 13 gm% in male & < 12 gm % in female, PBF showing microcytic hypochromic anaemia, MCV < 75 fl, S. Iron < 60 µg /dl,Serum Ferritin level < 15 µg/l,Serum transferrin saturation < 16 %.

### Exclusion Criteria:

Patient of chronic kidney disease (GFR < 60 ml/min/1.73 $m^2$), calculated by Cockcroft-Gault equation, Known case of diabetes mellitus or newly diagnosed diabetes mellitus, Hemolytic anemia (defined by reticulocyte production index > 2),Chronic alcoholic &Those unable to give informed consent.

**Control Group:**
Non anemic patients & Non- diabetic patients

**Statistical Analysis**:
Data were analysed using chi-square test, T-test, Carl Pearson correlation coefficient. We used SPSS version 16 for Windows® for statistical application. Statistical significance was set at *p* value ≤ 0.05.

**RESULTS:**
The characteristics of both groups study population are compared & shown in table no.1. Male **:** female ratio is 2:3 & mean age is 35±5 in both groups. A significantly lower serum iron level (15.99 ± 6.40µg/dl), lower % transferrin saturation (4.68 ± 1.99%) and lower serum ferritin (10.52 ± 2.91µg/dl) with significantly higher TIBC (338.55 ± 40.97µg/dl) were found in the case group participants compared to non-anemic subjects indicating presence of IDA (p<0.05). The mean FBS , PPBS are 78.71 ± 11.70 **;** 123.89 ± 9.15 respectively in control group & 78.63 ± 10.49 **;** 124.38 ± 10.14 respectively in case group. The difference was not statistically significant. (P >0.05).

The mean HbA1c level in IDA patients (5.97 ± 0.69%) was significantly higher (p value <0.05) than non anemic subjects (5.24 ± 0.49%). IDA patients (case group) had a significantly higher frequency (76/100 cases) of higher HbA1c (>5.5%) than non anemic subjects(control group )(32/100 persons) as shown in table no.1 & 2. The distribution of HbA1c according to different age group is shown in table 2 . The distribution of HbA1c level in patients having HbA1c ≥ 5.5 in control group is in lower range mainly in 5.5-5.8 (13/32 subjects ) while in case group this is in higher range 5.8-6.1 ( 22/76 patients) & ≥ 6.4 (31/76 patients) as shown in table no.3.

Correlation between iron indices & HbA1c was calculated as Pearson coefficient (r value) .Serum iron,( r is -0.159, p value >0.05, NS), serum ferritin ( r is - 0.067 , p value >0.05 , NS ) and serum transferrin saturation ( r is -0.186 , p value >0.05, NS ) are correlated negatively with HbA1c level in control group . In case group also serum iron (r is - 0.462, p value <0.05, sig ) serum ferritin (r is - 0.257, p value <0.05, sig.) % transferrin saturation (r is - 0.088, p value >0.05, NS ) are negatively correlated with HbA1c. Serum TIBC was positively correlated with HbA1c (r is 0.016, p value >0.05, NS) in control group but negatively correlated in case group ( r is - 0.014, p value > 0.05, NS ) but in both group observation was non-significant.

**Table 1: Characteristics of study participants**

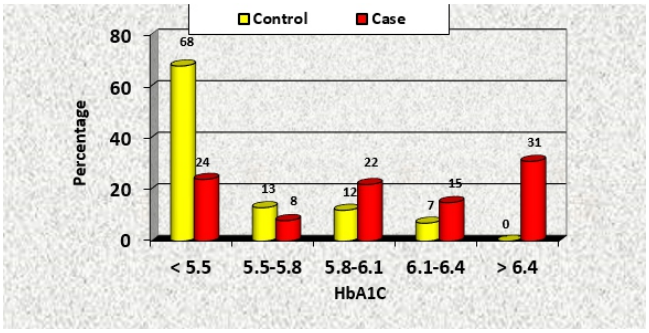
| Measured variable | | Study population | | P value | Significance |
|---|---|---|---|---|---|
| | | Control | Case | | |
| 1) Total no. of patients | | 100 | 100 | | |
| 2) Total no. of female patients | | 60 | 61 | | |
| 3) Total no. of male patients | | 40 | 39 | | |
| 4) Male/female | | 40/60 | 39/61 | >0.05 | NS |
| 5) Mean age (in years) | | 35±5 | 35 ± 5 | >0.05 | NS |
| 6) Mean Hb (gm/dl) | | 14.73 ± 0.89 | 7.33 ± 1.45 | <0.05 | S |
| 7) Mean MCV (fl) | | 88.46 ± 4.73 | 68.38 ± 2.53 | <0.05 | S |
| 8) Mean serum iron (µg/dl) | | 76.67 ± 12.19 | 15.99 ± 6.4 | <0.05 | S |
| 9) Mean serum TIBC (µg/dl) | | 263.41 ± 33.88 | 338.55 ± 40.97 | <0.05 | S |
| 10) Mean % transferrin saturation | | 29.38 ± 4.7 | 4.64 ± 1.92 | <0.05 | S |
| 11) Mean serum Ferritin (µg/l) | | 68.75 ± 13.68 | 10.52 ± 2.91 | <0.05 | S |
| 12) No. of patients having HbA1c level > 5.5 | | 32 | 76 | <0.05 | S |
| 13) No. of patients having HbA1c level <5.5 | | 68 | 24 | <0.05 | S |
| 14) Mean HbA1c level % | | 5.24 ± 0.48 | 5.97 ± 0.69 | <0.05 | S |
| 15) Mean HbA1c level of patients having HbA1c % | <5.5 | 4.97 ± 0.31 | 5.03 ± 0.37 | >0.05 | NS |
| | > 5.5 | 5.82 ± 0.22 | 6.27 ± 0.46 | <0.001 | HS |
| 16) Mean FBS (mg/dl) | | 78.71 ± 11.70 | 78.63 ± 10.49 | >0.05 | NS |
| 17) Mean PPBS( mg/dl) | | 123.89 ± 9.15 | 124.38 ± 10.14 | >0.05 | NS |

**Table 2: Age & Sex wise, HbA1c distribution of control & case group subject**

| Age group (in yrs) | Control | | | | | Case | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Male | Female | HbA1c <5.5 | HbA1c > 5.5 | Total | Male | Female | HbA1c<5.5 | HbA1c >5.5 | Total |
| 14-23 | 2 | 10 | 9 | 3 | 12 | 4 | 9 | 4 | 9 | 13 |
| 24-33 | 10 | 21 | 20 | 11 | 31 | 9 | 17 | 5 | 21 | 26 |
| 34-43 | 18 | 20 | 24 | 14 | 38 | 17 | 17 | 8 | 26 | 34 |
| 44-53 | 6 | 8 | 12 | 2 | 14 | 5 | 8 | 4 | 9 | 13 |
| 54+ | 3 | 2 | 3 | 2 | 5 | 5 | 9 | 3 | 11 | 14 |
| Total | 39 | 61 | 68 | 32 | 100 | 40 | 60 | 24 | 76 | 100 |

**Table 3: Distribution OF HbA1c in Control & Case Group Subjects**

| HbA1C | Control | | Case | |
|---|---|---|---|---|
| | No. | % | No. | % |
| < 5.5 | 68 | 68.00 | 24 | 24.00 |
| 5.5-5.8 | 13 | 13.00 | 8 | 8.00 |
| 5.8-6.1 | 12 | 12.00 | 22 | 22.00 |
| 6.1-6.4 | 7 | 7.00 | 15 | 15.00 |
| > 6.4 | 0 | 0.00 | 31 | 31.00 |
| Total | 100 | 100.00 | 100 | 100.00 |




**Graph no.1: Distribution of HbA1c level in control & case group**

**Table 4: Correlation between iron indices & HbA1c level**

| | Case group | | | Control group | | |
|---|---|---|---|---|---|---|
| | r | p | Sig. | r | P | Sig |
| s. iron | -0.462 | <0.05 | Sig. | -0.159 | >0.05 | NS |
| %transferrin saturation | -0.088 | >0.05 | NS | -0.186 | >0.05 | NS |
| S.ferritin | -0.257 | <0.05 | Sig. | -0.067 | >0.05 | NS |
| S.TIBC | -0.014 | >0.05 | NS | 0.016 | >0.05 | NS |

**DISCUSSION:**

**Age & sex distribution of the subjects:**

we tried to make equal proportion of gender & age to reduce bias. Coban et al in their study[12] included 50 subjects out of which 30 were women & 20 were men & mean age was 35.7 ± 11.9. Catherine et al in their study[16] included 6666 women out of which 1150 i.e. 13.7% were iron deficient. Among men ( n =3869) , only 75 i.e. 1.6% were iron deficient. Hashimoto et al included[14] women & mean age was 30.5 ± 4.1.Shanthi B et al[17] in their study included 50 iron deficient patients & 50 healthy subjects. Both groups were non-diabetic.

**Distribution of HbA1c Level in Case & Control Group:**

Catherin et al[16] in their study found that among iron deficient anemic, 27.4% of women & 17.33 % of men are having HbA1c level ≥5.5 & 10.1% of women participants & 1.33 % of men participants (who are having HbA1c >5.5) have HbA1c >6.5 . In result of their study they found that iron deficiency, a common condition among reproductive age women, was associated with shifts in HbA1c distribution to higher levels, but this shift occurred primarily between < 5.5 & 5.5-6.0 %.In our study, subjects of IDA are having HbA1c distribution pattern in higher range i.e. 5.8-6.1 (22% vs 12%); 6.1-6.4 (15% vs 7%) & ≥ 6.4 (31% vs 0% ) in comparison to non anemic patients. So shifting occurs in higher range.

**Mean HbA1c Level in Control & Case Group:** In our study mean HbA1c level of subjects having HbA1c >5.5 is higher in case group (6.27 ± 0.46) than in control group (5.82 ± 0.22) with highly significant (p value < 0.001). Mean HbA1c level of subjects having HbA1c < 5.5, is also higher in case group (5.03 ± 0.36 ) than in control group (4.96 ± 0.32) which is statistically significant (p value <0.05). Overall mean HbA1c level in control group was 5.24 ± 0.48 & in case group it was 5.97 ± 0.69 with p value <0.05. The mean FBS & PPBS were almost similar (78.71 ± 11.70 in control vs 78.63 ± 10.49 in case & 123.89 ± 9.15 in control vs; 124.38 ± 10.14 in case group ) with no significant difference in mean FBS & PPBS in case IDA & non anemic patients.

Catherine et al[16] found that mean HbA1c level in IDA women was significantly higher i.e. 5.32 in comparison to 5.27 (p value <0.05) in non anemic women, while in men there was no difference in mean level. After adjusting FBS, IDA was still associated with a greater mean level of A1C as well as a greater odds of having an A1C ≥5.5%. ( p value <0.05 ). After excluding previously undiagnosed the diabetic patients, greater odds of having HbA1c >5.5 was not significant.

Coban E etal[12] found that mean HbA1c level of IDA patients was 7.4 ± 0.8 while that in healthy subjects was 5.9 ±0.5 with significant p value. Patients who had glucose tolerance abnormalities (impaired glucose tolerance or diabetes mellitus), hemoglobinopathies, hemolytic anemia, chronic alcohol ingestion and chronic renal failure were excluded from the study. In patients with IDA, HbA1c decreased significantly after iron treatment from a mean of 7.4% +/- 0.8 to 6.2% +/- 0.6 (p < 0.001).

Shanthi B et al[17] in their study found higher mean HbA1c level 7.6 ±0.5 in IDA subjects who were non diabetic while mean HbA1c level in nonanemic non diabetic subjects was 5.5 ± 0.8 with significant p value < 0.001. There were no differences in the levels of fasting and postprandial glucose between the IDA and the control subjects (p > 0.05) .HbA1c is not affected by the blood sugar levels alone, and there are various confounding factors when HbA1c is measured, especially that of IDA, which is the commonest of the deficiency diseases worldwide.

Tarim et al[11] found that HbA1c in IDA patients decreased from 7.6 ± 2.6 to 6.2 ± 1.4% after iron therapy ($P < 0.05$), despite similar glucose levels.

**Correlation of Iron Indices With Hba1c Level :**

Our study observes iron indices to find correlation with HbA1C to specify association of IDA among anemia. We found that serum iron ( r value – 0.159 ,p > 0.05 ); % transferrin saturation (r value – 0.186 & p value > 0.05,) & serum ferritin ( r value -0.067 & p value > 0.05 ) were negatively correlated with HbA1c level in control group. In case group also serum iron ( r value -0.462, p value <0.01); % transferring saturation ( r value –0.088 with p value >0.05 ) ; serum ferritin ( r value -0.257 with p value <0.05 ) were negatively correlated to HbA1c .So negative correlation is more strong & significant with respect of serum iron & serum ferritin in IDA patients in comparison to non anemic patients. (Table no.4)

Previous studies of the influence of IDA and glucose control have documented the high prevalence of IDA in pregnancy[14] and the association with erythrocyte indices[18]. In a premenopausal non-pregnant population, Koga et al[18] found that red cell count is positively associated with HbA1c while haemoglobin, MCH shows negative correlation independent of plasma sugar level. Although in IDA patients, HbA1C is known to be elevated[12], It was recently found that HbA1C levels are also elevated in iron-deficiency states without anemia[18]. Hashimoto et al[14] found that A1C levels were significantly increased in the third trimester compared with earlier in pregnancy, but serum glycated albumin did not change; A1C was negatively correlated with serum ferritin and transferrin saturation, suggesting that A1C was influenced by iron stores rather than by glucose control. Furthermore, replacement with iron is associated with decreases in A1C, independent of glucose changes. Coban et al[12] found that among nondiabetic adults with iron-deficiency anemia, the A1C was 7.4 ± 0.3% before treatment and 6.2 ± 0.6% after treatment. Likewise, Tarim et al[11] found that A1C in iron-deficient patients decreased from 7.6 ± 2.6 to 6.2 ± 1.4% after iron therapy ($P <$ 0.05), despite similar glucose levels.

There are several proposed mechanisms for increase in haemoglobin glycation in IDA. One interpretation is that lipid peroxidation can cause increased glycation of hemoglobin[15] . Sundaram et al[13] also observed increase in plasma lipid peroxides in IDA patients and its correction with iron supplementation. In addition, a low level of antioxidants also stimulates formation of hemoglobinglycation[15]. Recently, it was observed that malondialdehyde, which is increased in IDA patients enhances the glycation of Hemoglobin.[13]

**CONCLUSION:**

In our study we concluded that mean HbA1c level was increased in iron deficiency anemic non diabetic patients in comparison to non anemic non diabetic patients independent of blood sugar level. Furthermore HbA1c level is shifted to higher range in iron deficient anemic patients. So it should be interpreted carefully in all iron deficient anemic patients for diagnosis & monitoring of diabetes mellitus.

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